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Replication timing and nuclear structure

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Abstract

DNA replication proceeds along spatially and temporally coordinated patterns within the nucleus, thus protecting the genome during the synthesis of new genetic material. While we have been able to visualize replication patterns on DNA fibers for 50 years, recent developments and discoveries have provided a greater insight into how DNA replication is controlled. In this review, we highlight many of these discoveries. Of great interest are the physiological role of the replication timing program, cis and trans-acting factors that modulate replication timing and the effects of chromatin structure on the replication timing program. We also discuss future directions in the study of replication timing.

Introduction

Duplication of the genetic material prior to each cell division requires stepwise detangling, unwinding, synthesis and reassembly of the entire length of nuclear DNA. Because this process necessarily endangers genomic stability, replication is highly coordinated spatially and temporally within the nucleus. Replication patterns of single DNA fibers were first visualized in mammalian cells half a century ago [1] and follow-up experiments detected DNA replication events as foci within interphase nuclei [2–4]. Both visualization approaches clearly indicate that DNA synthesis proceeds along temporal and spatial patterns that are correlated with nuclear structure. Advances in whole-genome analyses of replicating DNA continue to support the notion that nuclear DNA replicates with a distinct, consistent and often tissue-specific order (“replication timing”) that is strongly associated with spatial patterns of chromatin organization ([5,6]; for a recent review, see [7]).

In mammals, DNA replication starts at many sites (replication origins) on each chromosome. Those origins often cluster into larger domains called replication timing domains, each containing multiple origins that replicate concomitantly or within a short time window [8,9]. These domains vary in length from hundreds of kilobases to megabases [8–14]. Replicon clusters can be visualized as discrete foci of pulse-labelled DNA (replication foci) whose density, size and distribution vary as cells progress from early to late synthesis (S) phase of

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the cell cycle. Sequential pulse-chase experiments have demonstrated that the DNA sequences replicated at individual sites are consistent temporally and spatially as the cell progresses through the cell cycle and into subsequent generations [15].

Early replicating DNA often consists of regions of open chromatin that contain transcribed regions. As S-phase proceeds, early replicating domains complete replication, and DNA synthesis transitions gradually to replicate more condensed, less transcriptionally active chromatin in mid S-phase. Condensed, heterochromatic DNA often replicates in late S-phase (Figure 1). For example, centromeric cores, which contain centromeric heterochromatin, replicate in mid S-phase, before heavily compacted classical heterochromatin [16]. Genomic analysis of replication timing domains demonstrate that these domains are conserved within cells of any particular tissue type or developmental stage, and domain boundary analysis showed that replication domains highly colocalize with topologically associating domains (TADs), which are substructures located within chromatin compartments identified by high-throughput chromosome conformation capture (Hi-C) [12,17–20]. These studies imply that the order of DNA replication is associated with the same spatial features that constrain the distribution of chromatin in the interphase nucleus.

Because cells of different tissues of origin and developmental stages require specialized transcription programs, the ability to alter the order of DNA replication might confer an evolutionary advantage by facilitating a rapid adaptation to a changing chromatin landscape during differentiation. In support of this hypothesis, the order of replication changes in a large fraction of the human genome during nuclear reorganization processes associated with differentiation and development [21,22]. In this review, we will discuss the possible role of a replication timing program in metazoans and explore the known determinants of the order of replication in somatic cells with a special emphasis on how the replication program relates to the determinants of chromatin organization in the nucleus.

Physiological role of the replication timing program

The need for a consistent, developmentally and tissue-specific temporal order for eukaryotic genome duplication is not immediately evident. Entire genomes (for example, during the early embryonic stages in flies and frogs) can be assembled and rapidly duplicated without an apparently consistent order [23]. Consistent replication timing programs, however, appear to be ubiquitous in differentiated somatic cells and in embryonic cells after the activation of zygotic transcription, suggesting that the consistent order of DNA replication might facilitate genome integrity by coordinating replication with transcription and chromatin assembly on the shared chromatin template.

Two lines of evidence suggest that a consistent replication timing program plays a role in the maintenance of genomic stability by preventing conflicts between genome duplication and other enzymatic processes occurring concomitantly on chromatin, such as transcription. First, changes in replication timing are hallmarks of certain cancers [24–26] and also accompany pathological chromosomal rearrangements in several rare genetic disorders [27,28]. Second, a distinct order of DNA replication is primarily a property of the euchromatin component of nuclear DNA. In fact, the order of replication within extended

heterochromatin domains does not seem to be consistent. For example, an allele-specific chromosomally phased study of the replication timing program along the active and inactive chromosomes revealed that replication proceeded with a consistent order in the active X chromosome, whereas the order of replication within the inactive X varied among individuals and resembled a random, unstructured process [8,29]. Late-replicating, transcriptionally inactive regions on the autosomes also replicated in a similar manner, suggesting that in mammalian cells, inactive and condensed chromatin regions replicate rapidly in an apparently random order [29]. Furthermore, late replication of heterochromatin may preserve the structural integrity of the nucleus by preventing rapid, massive chromatin decondensation and re-condensation at the early stages of genome duplication [30]. Taken together with evidence for stochastic replication in cellular systems that do not activate transcription, such as during early embryogenesis [23], these observations imply that the temporal order of DNA replication facilitates coordination of replication with gene expression.

An additional role of the replication timing program, in particular the early replication of highly expressed genomic regions, might be to provide the necessary templates for transcription of genes that are typically expressed during S-phase. For example, transcription of the histone genes, which in most cells replicate early during S-phase, was halved when the replication timing of histone gene regions was delayed [31]. In addition, studies in induced human embryonic stem cell reveal that during differentiation, almost all the expressed genes, whether constitutive or differentiation-dependent, replicate at early S-phase. Only a very small fraction of expressed genes, which typically exhibit low levels of expression, replicate late [32]. These data suggest that DNA replication in early S-phase contributes towards maintaining high expression levels of genes actively transcribed during S-phase.

The mutation landscape of the genome is also influenced by replication timing. This influence is particularly apparent during carcinogenesis and genome evolution. Sections of DNA that replicate at different times during S-phase tend to have different mutation patterns. Point mutations and copy number losses correlate with late replication, whereas copy number gains and other rearrangements correlate with early replication (for a review, see [33]). Early replicating regions exhibit lower point mutation rates than regions replicating late, possibly because mismatch repair (MMR) efficiency is higher during early replication [34–36]. Higher MMR efficiency during early S-phase might therefor select for the replication of actively transcribed genes in early S-phase. Deregulated initiation of DNA replication on transcribed DNA increases the number of head-on collisions between DNA replication and transcription machinery. The collisions lead to R-loops and genome instability [37] that demonstrate the importance of coordinating replication timing and transcription.

Cis-elements as determinants of replication timing

Mechanistically, replication timing domains that replicate in a consistent order require the activation of groups of replication origins at distinct times during S-phase. The sequential activation of replication origins is evident within replication timing domains. For example,

in human basal erythroblasts, small highly reproducible peaks (Figure 1) are detectable within the well-characterized, megabase-sized replication timing domains correlate with clusters of replication origins detected by nascent strand analyses [8]. Experiments correlating the frequency of replication initiation events as measured by the direction of Okazaki fragments with large replication timing domains also support this conclusion [38].

Although epigenetic properties, such as histone modifications, play an important role in determining initiation frequency [10,39,40], replication origin activity is determined in part by the primary DNA sequence, as origin sequences can initiate DNA replication at ectopic sites [41–43]. Primary DNA sequence properties also correlate in part with replication timing. For example, early-replicating regions correspond to gene-rich, high-GC domains, whereas late-replicating regions correspond to gene-poor, low-GC domains [44]. However, replication timing does not correlate with either GC or repeat content in zebrafish [45], suggesting a link between DNA replication and functional aspects of the genome rather than sequence composition.

Replication origins may regulate chromatin and replication timing via specific DNA sequences that are easily unwound or by recruiting sequence-specific proteins that modify local and distal interactions on chromatin. Consistent with this suggestion, inherited variants that affect replication origin activity and replication timing have been identified in two experiments: analyses of replication timing in 161 samples of proliferating cells [46] and analyses of phased genomes, which permit identification of paternal or maternal origins to characterize the effects of specific sequence variations on origin activity [8,9]. In parallel, replication origins may also interact directly or indirectly with local or long distance cis-elements to modulate replication (see [47] for details). For example, an interaction between the replication origin and the locus control region at the human β-globin locus [48] affects the time of replication and is essential for both replication initiation and prevention of gene silencing [41,42,49].

In addition to sequences at replication origins, cis-acting sequences that modulate large chromatin domains can also affect the order of replication. For example, heterochromatin formation and replication delay can be modulated via non-coding RNAs such as XIST for the X chromosome [29,47] and ASAR6 and ASAR15 for the autosomal chromosome 6 and 15, respectively, in cancer cells [50,51]. These long non-coding RNAs, which remain associated with the chromosome territories from which they are transcribed, are implicated in the control of monoallelic gene expression but may be involved more broadly in chromosomal maintenance throughout the cell cycle [50,51]. Replication associated with ASAR6 transgenes requires an anti-sense transcript of an L1 retrotransposon, implicating L1 antisense RNA as a player in the regulation of chromosome-wide replication timing [51]. Cis-acting genetic elements at TADs can also determine, at least in part, the locations of megabase-scale replication timing domains. In cancer cells, the replication of entire chromosomes may be delayed in a sequence-specific manner, and interactions with long non-coding RNAs could alter the timing of replication for entire chromosomes [50].

Chromatin structure

The partial dependence on the primary sequence does not necessarily imply that specific sequences solely determine the timing of DNA replication, either directly or indirectly. In fact, recent studies using a combination of mathematical modelling and high-throughput genomics provide evidence to the contrary, suggesting that the distribution of replication origins by itself is sufficient to determine the order of replication initiation, and hence the replication timing program. For example, a mathematical model assuming a single rate-limiting factor for DNA replication and relying solely on DNase hypersensitivity data and the relative efficiencies of initiation at specific loci [52] accurately recapitulated cell-specific DNA replication timing patterns, including abnormal timing in cancer cells. This observation implies that it is possible to predict replication timing with high accuracy in human cells without assuming any “replication timing factor”. A second model [53], assuming spontaneous stochastic initiation within euchromatin and facultative heterochromatin, successfully predicted the three-dimensional spatial and temporal organization of replication events as well as the timing of replication based on higher chromatin organization. The density of the mini-chromosome maintenance (MCM) replicative helicase, assuming a high level of MCMs at early origins [54], was also able to predict the replication timing program, suggesting that the spatial distribution of replication origins determines the temporal organization of the replication process.

High-resolution whole-genome analyses have revealed that replication timing domains often reflect chromatin modifications [11,21]. Early replicating regions associate with transcriptionally active topological domains, whereas late replicating origins often associate with heterochromatin. The establishment of the replication-timing program occurs during the early G1 phase of the cell cycle, around the same time as TADs have been shown to assemble and become compartmentalized in mouse cells. The association between replication timing domains and TADs suggests that the timing of activation of replication origins reflects a fundamental structural property of the nucleus [17,19,21,55]. Replication origins are known to associate with nuclear structural features such as matrix attachment sites, scaffold attachment sites and stabilizing anti repressor elements [47,56] as well as with lamins and cohesins [39,47]. Replication origins also associate with chromatin modulators, such as a phosphorylated from of the histone deacetylase SIRT1 that prevents the initiation of DNA replication from a group of “dormant” origins [57].

The replication timing of specific genomic loci can vary with tissue type and differentiation status. For example, the human beta-globin locus replicates early during S-phase in erythroleukaemia cells whereas in non-erythroid cells it replicates later in S-phase [58], suggesting that other than sequence, chromatin environments also affect replication timing. Replication timing and initiation patterns tend to depend on cellular lineage rather than on cancer status in cancer cells [59]. Early replicating regions associate with open chromatin features, such as acetylation of H3K9, H3K18 and H3K27, methylation H3K4me and H3K36me3 [59]. Transcriptional activity coordinates with the replication timing program [32] and replication delays often accompany gene silencing. Origins that associate with open chromatin are activated in many cell types, and are enriched in moderately active transcription start sites [13,60]. In contrast, late replicating regions associate with closed

chromatin features, like hypoacetylation of H3 and H4, methylation H3K9 and H3K27, and they often initiate replication in a cell type specific manner [39,59].

Trans-acting factors

The binding patterns of pre-replication complexes do not provide clues to the principles of origin choice because the origin recognition complex, which anchors the pre-replication complexes to chromatin, does not bind specific DNA sequences [61]. Interactions between replication origins and components of pre-replication complexes, therefore, are essential for initiation but cannot provide a simple mechanistic explanation for the consistent replication patterns observed in most mitotic cell cycles.

The transcription factors Forkhead 1 (Fkh1) and Forkhead 2 (Fkh2) are required for clustering a subset of replication origins during G1 phase and for early initiation of these origins in S-phase in *S. cerevisiae* [62,63]. Fkh1 and Fkh2 promote early replication by recruiting replication factors. Fkh1 and Fkh2 regulate origin timing and establish changes in timing during the late G1 phase of the cell cycle [64], indicating that replication timing can be reset after origin licensing.

Distal DNA sequences modify transcriptional activity and origin activity through long-distance interactions [65–67]. Such interactions can be facilitated by chromatin remodelling factors, transcriptional activators and other proteins that bind enhancers and locus control regions [40,42,68]. For example, RepID, a protein that interacts with a group of replication origins, is associated with an origin-activating chromatin loop between the replication origin and the locus control region that facilitates early replication at the human beta-globin locus in erythroid cells [43].

Rif1, a shelterin component, is involved in regulating replication-timing in all eukaryotes studied so far by modulating the three-dimensional organization of the genome and recruiting protein phosphatase 1 (PP1). Rif1 interacts with G-quadruplex structures and recruits PP1 to modulate the chromatin binding of pre-initiation complex components [69–71] in genomic regions that undergo late replication. These actions might delay replication by preventing the essential replication kinase, DDK, from phosphorylating the replicating helicase (MCM2-7) while associating with nuclear architectural structures that anchor heterochromatin. Recent papers confirmed a direct interaction of Rif1 and PP1 in both human and mouse cells [72–74]. In fission yeast, a second Shelterin component, Taz1, binds along with Rif1 to heterochromatin-euchromatin boundaries to form heterchromatin compartments and regulate gene expression and replication timing [75,76].

The ORC-associated protein (ORCA/LRWD1), which stabilizes ORC on chromatin [77], temporally associates with late replication origins marked with H3K9me3 and methylated CpGs during G1 phase. In ORCA-depleted cells, levels of H3K9me3 and DNA methylation were altered at ORCA binding sites [78]. H4K20 tri-methylation mediated by Suv4-20h is necessary for the licensing and activity of some ORCA/LRWD1-associated origins. These origins ensure that late-replicating heterochromatin domains are replicated at the correct time [79].

Perspective and future directions: the role of the replication timing program

We have known for half a century that replication order follows a strict temporal and spatial organization, and recent studies on the whole-genome level have confirmed that replication patterns are tightly linked to nuclear architectural features. The challenge remains to tease out the determinants of replication timing, distinguish cause from effect, and address the biological questions associated with the replication timing program. An understanding of the association between late-replicating genomic regions and higher mutation rates would be particularly rewarding, as many genomic regions with recurrent mutations in cancer replicate late [46,80]. Lastly, the mechanism by which replication timing affects human genome sequence composition via GC-biased substitutions and gene conversions is an interesting subject of exploration [81–83].

Most studies measuring replication timing have been performed with cells growing in culture, in which the order of replication might reflect selective pressure for rapid proliferation. To understand the dynamics of replication timing more completely, especially the effects of changes in timing in cancer cells, replication timing will need to be investigated in patient samples. Such cells are hard to grow in vitro, but the development of xenografts in immunodeficient mice [84] has opened a novel avenue of research.

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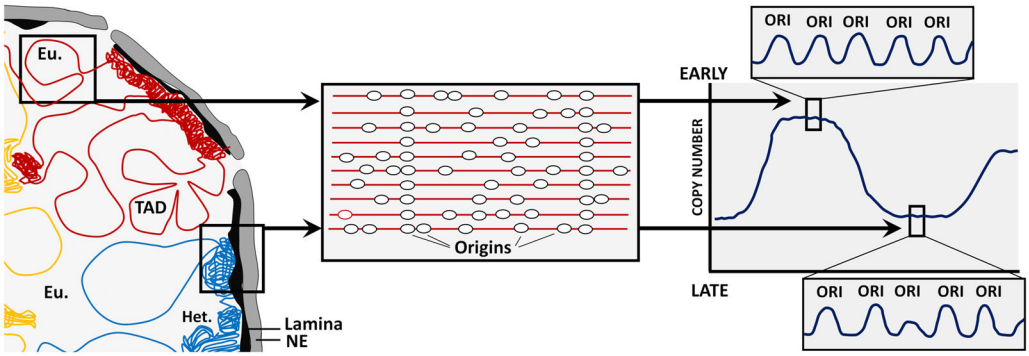

Figure 1.

Replication timing and nuclear structure: To co-ordinate DNA replication with transcription and chromatin assembly, chromatin inside the nucleus is organized into distinct replication timing domains. Replication timing domains are highly colocalized with topologically associating domains (TADs). Some domains contain euchromatin with actively transcribed genes and usually replicate at early S phase; some domains contain heterochromatin with silenced genes and replicate at late S phase. Inside each replication timing domain, many origins cluster together and initiate at similar times. Replication timing can be quantified by measuring relative copy number in proliferating cells. Replication timing domains can be detected as magabase-sized peaks when relative copy number is plotted, and replication origins can be detected as small peaks (ripples) within replication timing domains. Eu: euchromatin. Het: heterchromatin. Ori: origins. NE: nuclear envelope.

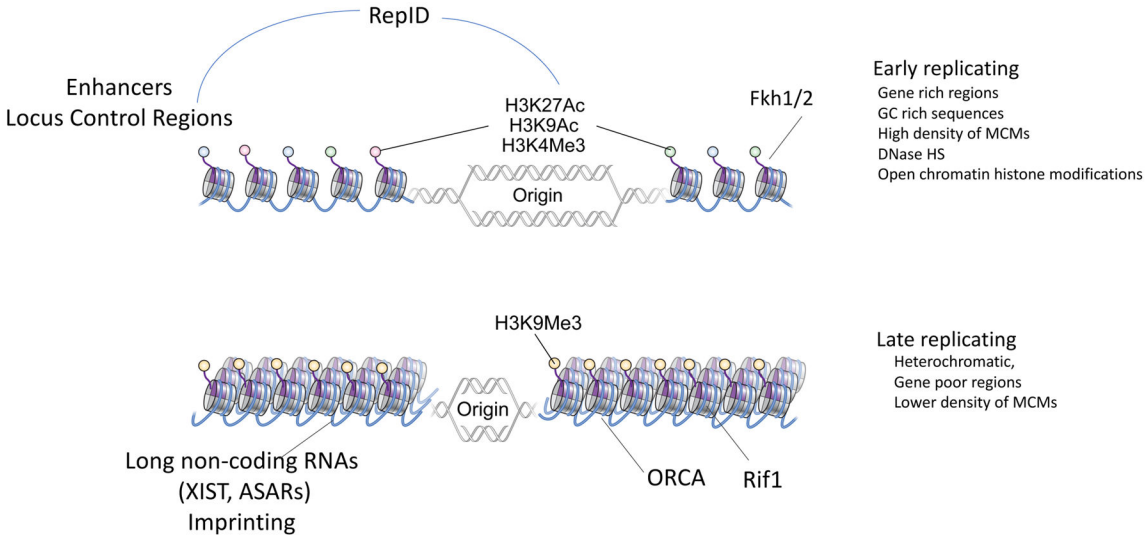

Figure 2.

Cis- and trans-factors establish early DNA replication domains with open chromatin and late DNA replication domains with heterochromatin. In addition to replicators and replication initiation complexes, other cis- and trans-factors affect nuclear structures and DNA replication timing. For example, RepID regulates DNA replication initiation by forming loops linking enhancer and/or locus control regions with replication origins; Fkh1/2 cluster groups of origins in the G1 phase for early replication in S phase; Rif1 and ORCA prevent late replicating regions from replicating earlier. Long non-coding RNAs can also affect chromatin structure and replication timing locally and mediate the action of distal regulators.